

Claims

We claim:

1. A recombinant bacterium engineered to secrete a cytokine and to express a tumor
5 antigen.
2. The recombinant bacterium of claim 1, wherein said bacterium is *E. coli*.
3. The recombinant bacterium of claim 1, wherein said bacterium is a
10 mycobacterium.
4. The recombinant bacterium of claim 3, wherein said bacterium is attenuated
Mycobacterium bovis, bacillus Calmette Guerin (BCG).
- 15 5. The recombinant bacterium of claim 1, wherein said cytokine is a Th1 cytokine.
6. The recombinant bacterium of claim 5, wherein said Th1 cytokine is selected
from the group consisting of GM-CSF, IFN- γ , TNF- α , IL-2, IL-12, IL-15 and IL-18
- 20 7. The recombinant bacterium of claim 6, wherein said Th1 cytokine is IL-2.
8. The recombinant bacterium of claim 1, wherein said tumor antigen is selected
from the group consisting of MUC1, CEA, oncofetal antigens and tumor-associated
antigens
- 25 9. The recombinant bacterium of claim 8, wherein said tumor antigen is MUC1.
10. A recombinant mycobacterium comprising a first nucleic acid molecule encoding
a Th1 cytokine operatively linked to a first promoter and a mycobacterial secretion
30 signal sequence and a second DNA molecule encoding a tumor antigen operatively
linked to a second promoter, wherein said cytokine is expressed and secreted from said
mycobacterium and said tumor antigen is expressed by said mycobacterium, such that

said recombinant mycobacterium is capable of inducing an immune response to said tumor antigen in a subject.

11. The recombinant bacterium of claim 10, wherein said first promoter is a bacterial
5 heat shock protein (hsp) gene promoter or a bacterial stress protein gene promoter.

12. The recombinant bacterium of claim 11, wherein said first promoter is hsp60 or
hsp70.

10 13. The recombinant bacterium of claim 10, wherein said second promoter is a
bacterial heat shock gene promoter or a bacterial stress protein gene promoter.

14. The recombinant bacterium of claim 13, wherein said second promoter is hsp60
or hsp70.

15 15. The recombinant bacterium of claim 10, wherein said secretion signal sequence
is the BCG alpha antigen signal sequence.

16. A recombinant mycobacterium comprising:

- 20 (a) a first DNA molecule encoding a Th1 cytokine;
(b) a first promoter;
(c) a mycobacterial secretion sequence;
(d) a second DNA molecule encoding a tumor antigen; and
(e) a second promoter;

25 wherein the 5' to 3' order is said first promoter of (b), said secretion signal sequence of
(c), said first DNA molecule of (a), said second promoter of (e) and said second DNA
molecule of (d), wherein the expression of said first DNA molecule of (a) is under the
control of said first promoter of (b) and said cytokine is expressed and secreted from
said mycobacterium and the expression of said second DNA molecule of (d) is under the
30 control of said second promoter of (e) and said tumor antigen is expressed by said
mycobacterium such that said recombinant mycobacterium is capable of inducing an
immune response to said tumor antigen in a subject.

17. A recombinant BCG having a dual promoter plasmid comprising:

- (a) a first DNA molecule encoding interleukin-2;
- (b) a first promoter;
- (c) a mycobacterial secretion signal sequence;
- 5 (d) a second DNA molecule encoding MUC1; and
- (e) a second promoter;

wherein the 5' to 3' order is said first promoter of (b), said secretion signal sequence of (c), said first DNA molecule of (a), said second promoter of (e) and said second DNA molecule of (d), wherein the expression of said first DNA molecule of (a) is under the control of said first promoter of (b) and the cytokine expressed and secreted from the mycobacterium and the expression of said second DNA molecule of (d) is under the control of said second promoter of (e) and the tumor antigen is expressed by said mycobacterium thereby inducing an immune response to said tumor antigen in a mammalian host.

18. A recombinant bacterium encoding a tumor antigen and having enhanced immunostimulatory properties comprising a first DNA molecule encoding a cytokine and a second DNA molecule encoding a tumor antigen, wherein said first DNA molecule encoding said cytokine is under the control of a first promoter and said cytokine is secreted from said bacterium in a biologically active form and wherein said second DNA molecule encoding said tumor antigen is under the control of a second promoter and said tumor antigen is expressed by said recombinant bacterium.

19. A recombinant BCG encoding MUC1 and having enhanced immunostimulatory properties and having incorporated therein a plasmid comprising a first DNA molecule encoding interleukin-2 operably linked to a first mycobacterial heat shock protein gene promoter and a mycobacterial secretion signal sequence and a second DNA molecule encoding MUC1 operably linked to a second mycobacterial heat shock protein gene promoter wherein the 5' to 3' order of said plasmid is said first promoter, said secretion signal sequence, said first DNA molecule encoding said interleukin-2, said second promoter, said second DNA molecule encoding MUC1 wherein said interleukin-2 is expressed and secreted from said recombinant BCG in a biologically active form and said MUC1 is expressed by said recombinant BCG.

20. An *E. coli*-BCG shuttle plasmid which, when expressed in a mycobacterium, results in specificity for a tumor antigen and enhanced immunostimulatory properties, said shuttle plasmid comprising:

- 5 (a) a first DNA molecule encoding a Th1 cytokine;
(b) a first promoter comprising DNA encoding a mycobacterial heat shock protein promoter and translational start site;
(c) a mycobacterial secretion signal sequence;
(d) a second DNA molecule encoding a tumor antigen; and
10 (e) a second promoter comprising DNA encoding mycobacterial heat shock protein promoter and translational start site;
wherein the 5' to 3' order is said first promoter of (b), said secretion signal sequence of (c), said first DNA molecule of (a), said second promoter of (e) and said second DNA molecule of (d), wherein the expression of said first DNA molecule of (a) is under the control of said first promoter of (b) and the cytokine is expressed and secreted from said
15 mycobacterium in a biologically active form and the expression of said second DNA molecule of (d) is under the control of said second promoter of (e) and the tumor antigen is expressed by said mycobacterium.

- 20 21. The *E. coli*-BCG shuttle plasmid of claim 20 further comprising an epitope tag 5' of said DNA encoding a Th1 cytokine.

22. The *E. coli*-BCG shuttle plasmid of claim 21, wherein said epitope tag is viral influenza hemagglutinin.

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23. The *E. coli*-BCG shuttle plasmid of claim 20 further comprising an epitope tag 5' of said DNA encoding a tumor antigen.

24. The *E. coli*-BCG shuttle plasmid of claim 23, wherein said epitope tag is viral
30 influenza hemagglutinin.

25. A method of inhibiting growth or proliferation of, or inducing killing of a tumor in a subject, comprising administering to said subject a recombinant bacterium of any

one of claims 1, 10, 16, 17, 18 or 19 in an amount that is effective to inhibit growth or proliferation of, reduce the size of, or induce killing of the tumor in the subject.

26. The method of claim 25, wherein said subject is a mammal

27. The method of claim 25, wherein said subject is a human.

28. A method of preventing the formation of a tumor in a subject comprising administering to said subject a recombinant bacterium of any of claims 1, 10, 16, 17, 18 or 19 in an amount that is effective to prevent the formation of said tumor in said subject.

29. A method for stimulating an immune response to an immunogenic protein or fragment thereof, in a subject, comprising administering an effective amount of a recombinant bacterium of any one of claims 1, 10, 16, 17, 18 or 19 such that an immune response is stimulated against said immunogenic protein in said subject.

30. A method of treating a subject with cancer comprising administering to said subject an effective amount of the recombinant bacterium of any one of claims 1, 10, 16, 17, 18 or 19 such that said cancer is treated.

31. The method of claim 29, wherein said cancer is selected from the group consisting of breast cancer, prostate cancer, colon cancer, lung cancer, pancreatic cancer and ovarian cancer.

32. The method of claim 31, wherein said cancer is breast cancer.

33. A vaccine for immunizing a subject against a neoplastic disease, comprising a recombinant bacterium of any one of claims 1, 10, 16, 17, 18 or 19 and a pharmaceutically acceptable carrier therefor, wherein the recombinant bacterium in an amount effective to immunize a subject against a neoplastic disease.

34. A pharmaceutical composition comprising a recombinant bacterium of any one of claims 1, 10, 16, 17, 18 or 19 and a pharmaceutically acceptable carrier.

35. A kit for immunizing a subject against a neoplastic disease comprising the
5 vaccine of claim 33 and instructions for use.

36. A kit for treating cancer in a subject comprising the pharmaceutical composition of claim 34 and instructions for use.